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Prevalence ratio of free fatty acid in obese group with non-alcoholic fatty liver disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is liver disorders characterized by macrovesicular fatty liver, fibrosis, cirrhosis that not associated with alcohol consumption. The prevalence of NAFLD has risen with a pandemic of obesity. The increase of free fatty acid (FFA) oxidation will induce endoplasmic reticulum stress that cause mitochondrial dysfunction and lead to increase reactive oxygen species (ROS) production causing apoptosis of liver cells. The aim of study was to determine the prevalence of FFA in the obese group. This was an observational analytical study with cross-sectional design to determine the prevalence ratio of FFA in the obese group with NAFLD compared to the group without NAFLD. Obese women who fulfill the inclusion and exclusion criteria were involved in this study. Five mL venous blood sample was collected for the measurement of lipid profile, liver enzyme and FFA. Fatty liver was evaluated using abdominal USG. The Chi-square test was used to analyze different proportions of FFA between the both groups. Sixty four subjects were participated in this study and classified into obese with NAFLD (39 subjects) and obese without NAFLD (25 subjects). The prevalence ratio of FFA with cutoff value ≥2.66 nmol/mL in the obese group with NAFLD was 4.3 times higher than those without NAFLD (95% IC: 3.5 – 42.3; p<0.001).

ABSTRAK

Non-alcoholic fatty liver disease (NAFLD) adalah gangguan hati yang ditandai dengan *macrovesicular fatty liver*, fibrosis, sirosis dan tanpa dikaitkan dengan konsumsi alkohol. Prevalensi NAFLD meningkat bersama pandemik obesitas. Peningkatan oksidasi asam lemak bebas (FFA) akan menginduksi stres retikulum endoplasma dan mengakibatkan disfungsi mitokondria yang menyebabkan peningkatan produksi spesies oksigen reaktif (ROS) dan terjadinya apoptosis sel-sel hati. Penelitian ini bertujuan untuk menghitung prevalensi FFA pada kelompok obesitas dengan NAFLD. Wanita obesitas yang memenuhi kriteria inklusi dan ekslusi dilibatkan dalam penelitian ini. Lima mL sampel darah vena dikumpulkan untuk pengukuran profil lipid, enzim hati dan FFA. Lemak hati diukur dengan USG pada bagian abdomen. Uji Chi-kuadrat digunakan untuk menganalisis perbedaan proporsi dari FFA antara grup obesitas dengan NAFLD dan grup obesitas tanpa NAFLD. Sebanyak 64 subjek berpartisipasi dalam penelitian ini dan dikelompokkan dalam grup obesitas dengan NAFLD (39 subjek) dan grup obesitas tanpa NAFLD (25 subjek). Rasio prevalensi FFA dengan nilai *cut off* ≥2,66 nmol/mL pada kelompok obesistas dengan NAFLD 4,3 kali lebih tinggi dari kelompok obesistas tanpa NAFLD (IK 95%: 3,5 – 42,3; p<0,001).

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INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is known as one of the chronic liver disorders in the developing countries with the prevalence of 10% - 24%.[1] Wilson *et al.*[2] reported that this disease is strongly correlated to obesity.[2] The highest prevalence of NAFLD was reported at 40-49 years old.[1] The NAFLD may develop into fibrosis or hepatic cirrhosis (15-50%) and mortality (10%).[3] Total patients with NAFLD, 5% of them has been developing to be hepatic cirrhosis in period of 7 years and 1.7% died caused by the hepatic cirrhosis.[4]

The consensus of NAFLD/Non Alcoholic Steatohepatitis (NASH) in Cebu reported that Indonesia has the highest number of sufferers of NAFLD in Southeast Asia at 30%, while, Malaysia and Singapore were only 17% and 5%. In accordance with the Household Health Survey in Indonesia in 2001 and 2004, there was increased prevalence of overweight (body mass index/BMI$\geq$ 25 - <30) from 11.1-15.5% and obesity (BMI$\geq$30) from 2.4% to 3.4%.[5]

Obesity is a state of excess fat accumulation in the body by fatty tissue and can cause several diseases. The occurrence of obesity in the world is increasing as results of the lifestyle modernization with increase calories, limited physical activities and urbanization also determined by environmental factor.[6]

There is a strong relation between NAFLD and obesity. Obesity is differentiated between central obesity (visceral) and peripheral obesity. The central obesity is the fat accumulation in both subcutaneous abdomen and intra-abdominal abdomen and also known as abdominal obesity. The peripheral obesity is the fat accumulation tends to be in the lower part of the body in gluteofemoral area.[7] Examination using abdomen CT-scan showed that the central obesity plays a very essential role towards the insulin resistance. This is because the central obesity-related to increasing FFA as a factor of insulin resistance.[8]

The increase of FFA oxidation will induce endoplasmic reticulum stress that activate the course of inhibitory kappa β kinase/nuclear factor kappa β(IKK-β/NF-kβ) lead to increase of inflammatory cytokine expression such as TNF-alpha, interleukin-6 (IL-6), interleukin-1β (IL-1β) and activation of Kupffer cells. The increase of FFA also cause of mitochondrial dysfunction and increase of reactive oxygen species (ROS) production causing the apoptosis of the heparin cells. The accumulation of inflammatory mediators in liver and apoptosis liver cell can cause non-alcoholic steatohepatitis (NASH). Other factors that contribute to increased FFA in plasma are increased sensitivity of fats deposit in the body to effect of catecholamine lipolysis and decrease sensitivity to the effect insulin antilipolysis on fat tissues that are insulin dependent and the bluntness of esterification of FFA relying on insulin in fat tissue.[9] This study was aimed to detrmine the prevalence ratio of FFA in the obese group with NAFLD compared without NAFLD.

MATERIALS AND METHODS

This study was conducted from August 2015 to March 2016. The protocol of the study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta. It was an analytical observational with cross-sectional design. The subjects were obese women from an Islamic Study Group, which were voluntarily participating in this study from Mantup hamlet, Baturetno sub-district, Gemawang hamlet and Rejodani hamlet, Yogyakarta Special Region, Indonesia. Subjects were selected in order to fulfil the inclusion and exclusion criteria. The inclusion criteria included the female aged $\geq$17 years old, obese based on Pacific Asia female criteria in accordance with WHO with BMI $\geq$25 kg/m^2 diagnosed by USG and showed NAFLD and without NAFLD,

no record for the consumption of ≥20 g alcohol/day (2 glasses/day), agreed and signed the informed consent. The exclusion criteria included the patient with the history of hepatitis and other diseases that had the equal display of USG such as drastic weight reduction, drug consumption caused steatosis such as steroid, estrogen, calcium-blocker, amiodarone, tetracycline, tamoxifen, methotrexate, valproic acid, cocaine and antivirus.

The measurement of FFA level was conducted using the quantitative method of sandwich enzyme immunoassay (Qayee-Bio, Shanghai, China), lipid profile using automatic chemistry analyzer dimension EXL 200 (Siemens, Germany). The subjects who fulfilled the inclusion and exclusion criteria were asked to attend at Clinical Laboratory Installation of Dr. Sardjito General Hospital, Department of Clinical Pathology and Medical Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta and Installation of Radiology, Academic Hospital, Universitas Gadjah Mada, Yogyakarta at the time agreed during the period of specified study.

Data analysis

The data of the characteristic of the research subject were presented descriptively or as mean and standard deviation (SD) if the distribution was normal, median and maximum-minimum value if the distribution was not normal. The continual test of the normality used Kolmogorov Smirnov. The test on the difference between the two groups was conducted using independent t-test or Mann Whitney U-test. The analysis of different proportion was conducted using Chi-square by measuring the ratio of the prevalence (RP) with 95% CI, $p<0.05$. The analysis with the curve of the receiver operating characteristic (ROC) was done to determine the cut off value of FFA level. The area under curve (AUC) is an area under the curve of ROC that can be used to assess the accuracy of a diagnosis.

RESULTS

The total of the subjects involved in this research was 64 people divided into the obese group with NAFLD (39 people) and the obese group without NAFLD (25 people) (TABLE 1).

TABLE 1. The characteristics of the subjects in the obese group with and without NAFLD

Variable	Obese with NAFLD	Obese without NAFLD	p
Age (mean ± SD years)**	49.12 ± 10.74	49.9 ±11.83	0.79
Abdominal circumference [median (min-max) cm] *	103 (101.9-106.5)	103 (101.4–106.9)	0.89
Body Height (mean ± SD cm)**	153.32 ± 6.60	152.37 ± 6.92	0.59
Body Weight (mean ± SD kg)*	73.80 ± 13.47	70.90 ±10.00	0.38
BMI (mean ± SD kg/m2)*	31.40 ± 4.36	30.39 ± 2.94	0.34
Blood pressure [median (min-max)]			
• Systolic (mmHg) *	128 (121.71-130.59)	126 (121.48-135.78)	0.75
• Diastolic (mmHg) *	85 (81.98-88.78)	82.72 (78.2-87.5)	0.36

Note: *Mann Whitney U test; ** Independent t test

A significant difference was found in the triglyceride and FFA between the group of obese with NAFLD rather than in the one without NAFLD. There was no significant difference regarding age, body height, body weight, BMI, abdominal circumference, systolic blood pressure, diastolic, AST, ALT, total cholesterol, HDL cholesterol and LDL cholesterol in the obese group with NAFLD and without NAFLD (TABLE 2).

The receive operation curve (ROC) determined cut off levels of FFA was 2.66 nmol/mL by the area under curve (AUC) value of 0.78. At the cut off value was obtained a sensitivity of 80% and a specificity of 64%.

TABLE 2. Profile of the liver function test on obese group with and without NAFLD

Variable	Obese with NAFLD	Obese without NAFLD	p
FFA (mean ± SD nmol/mL)*	3.22 ± 0.69	2.50 ± 0.60	<0.001
AST [median (min-max) IU/L]*	30(16-40)	21(12-45)	0.07
ALT (mean ± SD IU/L)**	26.87±11.59	19.95±7.37	0.01
Total cholesterol [median (min-max) mg/dL]*	232(195-312)	252(174-320)	0.44
HDL cholesterol [median (min-max) IU/L]*	40(18-59)	36(25-68)	0.9
LDL cholesterol (mean ±SD mg/dL)**	191.18±51.33	173±37.68	0.16
Triglyceride cholesterol [median (min-max) mg/dL]*	197(132-388)	145(103-248)	0.001

Note: *Mann Whitney U test; ** Independent t test

The prevalence ratio of the increase of FFA in this research was 4.3 (2.1 – 21.7) with p value <0.0001. It can be interpreted that the increase of FFA ≥2.66 nmol/mL had the risk of 4.3 times higher than the occurrence of NAFLD.

From the population of samples studied, the confidence interval 95% (CI 95%) was between 2.1 – 21.7, thus the increase of FFA can be used as a good indication of the occurrence of NAFLD on the obese population (TABLE 3).

TABLE 3. The prevalence ratio of the increase of FFA on NAFLD of obese population

	Prevalence ratio	95% CI	p
Increasing FFA	4.30	2.1 – 21.7	<0.0001

DISCUSSION

NAFLD is indicated with the accumulation of the triglyceride, formed from the esterification of FFA and glycerol in the hepatocyte. FFA emerged in the liver from three different sources; those are lipolysis (hydrolysis of FFA and glycerol from triglyceride) in the adipose tissues, food sources and de novo lipogenesis. The genetic factor contributed to the regulation of de novo lipogenesis for the existence of the mutation in the nuclear transcription factor.[10]

Steatosis associated with chronic liver inflammation, partly mediated by activation of IKK-β/NF-κβ signaling. Increased activity of NF-κβ associated with liver expression of inflammatory cytokines such as TNF-α, IL-6 andIL-1β, and activation of Kupffer cells.[11] Pathway of IKK-β / NF-κβ in hepatocytes can also be activated directly by the FFA, further mechanisms of central obesity increased supply of FFA liver can cause inflammation. In addition, the data suggest that inflammation and activation of NF-κB can trigger carcinogenesis and chronic inflammation associated with hepatic steatosis can play a key role in the development of carcinoma hepatosellular.[11]

Ruhl *et al.*[12] reported that individuals with obesity/overweight and central fat distribution will evolve into NAFLD. The elevated of the prevalence of NAFLD consistent with an increasing of BMI. The results showed systolic and diastolic blood pressure to be higher in the obese group with NAFLD. The population of NAFLD with diabetes compared with NAFLD population without diabetes, p value of systolic and diastolic consecutive participated 0.417 and 0.709, although not found a statistically significant difference. Increased blood pressure is strongly associated with obesity and insulin resistance.[13]

Cytokines produced by adipocytes to stimulate lipolysis, which will lead to increased synthesis of FFA. Increased FFA cause an increased in production of triglycerides by the liver and increase hepatic VLDL secretion. Triglycerides increased in circulation resulting lipoprotein bring more triglycerides and less HDL.[14]

This study was obtained difference of mean levels of FFA in subjects NAFLD compared without NAFLD. These results are consistent with previous studies conducted by Purnama *et al.*[15] in which the FFA level in the obese group with diabetes mellitus (0.996±0.296 mE) higher than the obese group without diabetes mellitus (0.567±0.122 mE).[15]

In this study the ALT levels increased in the obese group with NAFLD compared without NAFLD. This is similar to research conducted by Paola *et al.*[16] ALT is more specific than AST to predict liver damage. A marker AST/ALT ratio as a screening occurrence of NAFLD prevalence is very low with the result of 15-20%, but if AST/ALT combined with abdominal ultrasound will produce a higher prevalence of NAFLD (20-46%).[17]

The usual observed biochemical pattern in hepatic steatosis due to NAFLD is of increased levels of transaminases, with ALT levels exceeding those of AST. This classical pattern is particularly useful in differentiating between hepatic steatosis from NAFLD and alcoholic liver injury, with the latter normally associated with a high AST:ALT ratio. The progression of hepatic steatosis to NASH and associated hepatic fibrosis, however, AST levels increase with a resultant rise in the AST:ALT ratio.[18] The degree of improvement in aminotransferase levels cannot be used as a predictive factor. Although in some cases the levels of ALT are higher than levels of AST, AST levels may be higher than ALT levels, especially when there cirrhosis.[19] Research conducted by Bellentani 55% of patients with NAFLD had normal aminotransferase levels. This shows that the liver enzyme is not a good marker for the diagnosis of NAFLD.[20]

The result of this study different from research conducted by Purnama *et al.*[15] subject of NAFLD with and without DM obtained OR 12.391 CI 95% with

p<0.001. The obese subject also suffered DM, it would increase the prevalence of the occurrence of NAFLD. It was because of the resistance of the insulin causing the occurrence of the increase of FFA.

The limitations of the study such as the width of CI 95% 2.1 to 21.7. It is probably caused by visceral fat distribution varies, the measurement of visceral fat levels can be done by checking dual X-ray absorptiometry (DXA) scan, this examination is expensive and only certain hospitals that have such facilities. Another limitation of this study, was not examined genetic factors as well as physical activity. Genetic testing of the genes that affect the regulation of de novo lipogenesis due to mutations in the nuclear transcription factor. Some of these receptors, among others sterol regulatory element-binding factor (SREBF) 1, liver X receptor (LXR) alpha, farnesoid X receptor (FXR), peroxisome proliferator-activated receptors (PPAR) gamma, PPAR Alpha. In additional the design of this study is still a cross-sectional design so it cannot explain the temporal relationship (causal), this can lead to bias because the data was only describing the current state of research.

CONCLUSIONS

The prevalence ratio of FFA in the obese group with NAFLD significantly increased 4.3 times compared to the group without NAFLD.

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